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In re Application of:

JOEL S. GREENBERGER, ET AL.

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Patent Examiner:

J. Souaya

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METHOD AND APPARATUS
FOR HOLDING CELLS

Pittsburgh, Pennsylvania 15213

CERTIFICATE OF MAILING

April 5, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

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AMENDMENT

In response to the Office Action dated October 5, 2001, please enter the
following amendments to the above-identified application as follows:

IN THE CLAIMS:

1. An apparatus for incubating and determining the state of individual cells
within a plurality of cells comprising:

a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell of the plurality of cells can be individually examined over time while the environment is dynamically controlled and maintained in the desired condition; and

a mechanism for automatically determining the state of said individual cell over time, said determining mechanism in communication with the incubating mechanism while the environment is dynamically controlled and maintained in the desired condition, said determining mechanism in communication with the incubating mechanism.

47. An apparatus as described in Claim 124 wherein the imaging mechanism includes a mechanism for phase contrast imaging to identify the state of said individual cell over time.

51. An apparatus for incubating and determining the state of individual cells within a plurality of cells comprising:

a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell can be examined in real time over time while the environment is dynamically controlled and maintained in the desired condition; and

3
a mechanism for determining the state of said individual cell in real time over time, said determining mechanism in communication with the incubating mechanism while the environment is dynamically controlled and maintained in the desired condition.

52. An apparatus as described in Claim 51 wherein the determining mechanism includes a mechanism for determining a biological event in said individual cell.

57. An apparatus for incubating and determining the state of individual cells within a plurality of cells comprising:

4
a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell of the plurality of cells can be examined over time while the environment is dynamically controlled and maintained in the desired condition; and

a mechanism for automatically tracking and identifying division and differentiation of said individual cell over time, said incubating mechanism in communication with the tracking and identifying mechanism.

✓ ✓
Cancel Claims 65-69.

70. An apparatus for incubating and determining the state of individual cells within a plurality of cells comprising:

15 a mechanism for incubating a first cell and at least a second cell amongst the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which the first cell and at least the second cell can be individually examined over time amongst the plurality of cells while the environment is dynamically controlled and maintained in the desired condition;

a mechanism for individually controlling the division and differentiation of the first cell and at least the second cell amongst the plurality of cells while the cells are in the incubating mechanism, said controlling mechanism controls the division and differentiation of the first cell differently from the way it controls the division and differentiation of the second cell amongst the plurality of cells while the cells are in the incubating mechanism; and

a mechanism for individually tracking and identifying division and differentiation of the first cell and at least the second cell amongst the plurality of cells over time.

~ ✓
Cancel Claims 71-73.

74. An apparatus for incubating and determining the state of a stem cell within a plurality of cells comprising:

a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell of the plurality of cells can be individually examined over time while the environment is dynamically controlled and maintained in the desired condition;

6
a mechanism for automatically determining a desired state of the stem cell within the plurality of cells, the determining mechanism connected to the incubating mechanism; and

a mechanism for introducing quiescence media to the stem cell within the plurality of cells in the incubating mechanism when the cell is in the desired state to inhibit the proliferation or selected differentiation of the cell, said introducing mechanism connected to the incubating mechanism.

75. An apparatus for incubating and determining the state of individual cells within a plurality of cells comprising:

6
a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell of the plurality of cells can be individually examined over time while the environment is dynamically controlled and maintained in the desired condition, said incubating mechanism having a mechanism for controlling the environment about said individual cell over time in the incubating mechanism to maintain the environment about said individual cell over time in a desired condition; and

a mechanism for determining the state of said individual cell over time, said determining mechanism in communication with the incubating mechanism.

80. An apparatus for incubating and determining the state of individual cells within a plurality of cells comprising:

7
a mechanism for incubating the plurality of cells having a dynamically controlled environment, which is maintained in a desired condition and in which each individual cell of the plurality of cells can be individually examined over time while the environment is dynamically controlled and maintained in the desired condition; and

a robotic mechanism including a robotic arm for dispensing and aspirating different material, a mechanism for controlling the environment about said individual cell over

D²
time within the plurality of cells in the incubating mechanism to maintain the environment about said individual cell over time within the plurality of cells in a desired condition.

✓ ✓
Cancel Claims 82-85.

✓ ✓
Cancel Claims 101 and 102.

✓ ✓
Cancel Claims 105-113.

Please add the following claim.

D⁸
124. An apparatus as described in Claim 1 wherein the determining mechanism includes an imaging mechanism which individually images said individual cell over time of the plurality of cells in the incubating mechanism.



REMARKS

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Claims 1, 47-64, 70, 74-81, 86-100, 103, 104 and 114-124 are currently active.

Claims 65-69, 71-73, 82-85, 101, 102 and 105-113 have been canceled in view of the finality of the restriction requirement.

Claim 124 has been added. Antecedent support for Claim 124 is found in Claim 1.

The Examiner has rejected Claims 57, 74, 80, 86-88, 90, 93, 99, 100, 114, 115 and 118-123 as being anticipated by Maruhashi. Applicants respectfully traverse this rejection.

Referring to Maruhashi, there is disclosed a method and apparatus for investigating and controlling an object. Maruhashi teaches there is a relationship between shapes of cells and the activities of cells. See column 5, lines 58-69. By identifying the different shapes and counting the number of respective shapes in the sample, the ratio of secreting cells can be extrapolated and obtained for an entire population. See column 6, lines 18-20. Based upon the recognition of the shape of the cell being indicative of the activities of the cell, Maruhashi teaches a image recognition control system. The system is comprised of a

tank 10 which contains liquid containing living bodies in suspension. Maruhashi teaches that a part of the liquid is withdrawn from the tank 10 and passed to an image pick-up device 20. An image of the part of the liquid is taken. Whatever cells are in the part are in the image but there is no control over which cells are in the part of the liquid. For any cell to actually be in the part is by chance and is arbitrary. This is because Maruhashi is concerned with large numbers of cells and what happens generally to all the cells, not "each" cell, or even any one cell "over time". It isn't possible for Maruhashi to monitor even one cell over time since the cells become lost in the liquid with the other cells. There is no teaching to mark or identify even one cell in Maruhashi to be able to monitor its state over time. The image pick-up device optically magnifies the image and stores it electrically as an image from a television camera. See column 8, lines 35-46. Maruhashi teaches that there is a transparent container 21 formed of glass or plastic in which the subject liquid is fed for observation from the liquid tank 10 to the container. See column 81, lines 58-62.

In another embodiment, Maruhashi teaches that a driving signal issues from a timer 33 causing liquid containing cells in suspension to be sampled and fed to the image pick-up device 20. See column 9, lines 14-18. In yet another embodiment, Maruhashi teaches a culture liquid sampled from a cell culturing tank is fed to image pick-up devices 26 and 27. The image pick-up device generates picture emerges for observing cells. See column 10, lines 20-25. In another embodiment, Maruhashi teaches that the culture liquid is sampled from the cell culturing tank 10 and fed to an image pick-up device 20 where pictures of the cells are

taken. See column 11, lines 4-9. In another embodiment, Maruhashi teaches a timer 33 generates driving signals which drive a pump of an image pick-up device 20. A culture liquid is sampled from a cell culture tank 10 and fed to an image pick-up device 20 where pictures are taken of the cells. See column 11, lines 26-33. In still another embodiment, Maruhashi teaches that the culture of liquid is sampled from the cell culture tank 470 and fed to an image pick-up device 420 where images of the culture of liquid are taken. See column 15, lines 27-33. In another embodiment, Maruhashi teaches a timer generates drive signals which drives the pump of the image pick-device 420. The culture liquid is sampled from the cell culture tank 470 and fed to the image pick-up device 420 where pictures are taken of the images. See column 15, lines 53-60. In yet another embodiment, Maruhashi teaches a pump 507 is operated to separate broth from the culture vessel 506 via the cell separator 505 and the duct 504, and separated broth is then passed to a collecting tank 508. An image pick up device 509 monitors the cells, where the image pick up device 509 operates in like manner corresponding to that described above for the earlier embodiments. See column 16, lines 32-39.

There is no teaching or suggestion of examining any single cell over time or the ability to examine any cell over time and know the cell had been previously imaged.

Maruhashi teaches in lines 18-53 of column 16 only that the broth and batches of cells are in the culture vessel and many cells at once are imaged. However, there is no teaching about identifying an individual cell, or how one cell could be examined over time in the midst of all the others cells in the same culture vessel to determine any single cell's state over time.

The goal of the apparatus taught by Maruhashi, as described in column 8, is to increase concentration of active living bodies to be useful in production systems for activated sludge and sewage treatment. In column 11, the apparatus is described to measure density of cells in liquid. In column 16, the final paragraph, it is described as being applicable to the flame in a furnace! All this also evidences that Maruhashi is only interested and only capable of macroscopic attributes formed from smaller units, but is not interested in the smaller units themselves over time.

While Maruhashi is composed of different separate components, they are connected together and could be considered one system. However, in regard to the claims, as amended, with the limitation of monitoring the cell over time, all the teachings of Maruhashi which require a part of the liquid to be moved evidence the fact that no cell can be monitored over time. This is because the cells are all intermingled with each other as they are transferred to the image device and then back to the tank. Additionally, the fact that parts of the liquid are transported with many cells but not individual cells or one cell at a time also evidences that Maruhashi cannot monitor an individual cell over time to identify its state. In fact, the need to transfer parts of the liquid with many cells so an image of many cells can be taken at one time to obtain a representative sampling of the state of all the cells in the tank actually teaches away from monitoring a single cell over time. This is because monitoring a single cell over time would require many individual cells to be monitored to yield the same result, which Maruhashi has no capability to perform.

Applicants' Claim 57, as amended, has the limitation of a "mechanism for incubating the plurality of cells . . . in which each individual cell of the plurality of cells can be examined over time . . . ; and a mechanism for automatically tracking and identifying said individual cell over time . . .". Maruhashi only teaches to view groups of cells at a time by an image-pick up device. Claims 74, 80, 86-88, 90, 93, 99, 100, 114, 115 and 118-123 are patentable for similar reasons over Maruhashi.

The Examiner has rejected Claims 57, 74, 80, 86-88, 90, 93, 99, 100, 114, 115 and 118-123 as being unpatentable over Matsuzaki. Applicants respectfully traverse this rejection.

Referring to Matsuzaki, there is disclosed an apparatus and method of culturing and diagnosis of animal cells using image processing. Matsuzaki teaches that the size of cells indicates whether they are living cells, dead cells and division potential-possessing cells. See column 4, lines 37-52. Matsuzaki teaches an apparatus comprises a culturing vessel 1, a culture medium observing portion 2, means 3 of introducing a culture medium to the observing portion 2, a measuring device 4 to measure sizes of cells in the observing portion 2, an analyzer 8 to determine a proportion of division potential-possessing cells or a portion of living cells on the basis of a cell size distribution, and means 6 to return a culture medium in the observing portion 2 to a culture medium in the culturing vessel. Figure 3 shows that the observing portion 2 is separate and apart from the culture vessel 1. Matsuzaki teaches the

culture medium in the culturing vessel is partially fed to the observing portion 2, and cells in the partially fed culture medium in the observing portion 2 are measured by the measuring device 4 for a cell size distribution. When the measurement is finished, the culture medium in the observing portion 2 is returned to the culturing vessel 1. See column 5, lines 23-47.

Matsuzaki teaches the culture medium is intermittently fed from the culturing vessel 1, to the measuring portion 2. The culture medium can also be measured while it is fed continuously to the measuring portion 2. See column 5, lines 52-56.

Matsuzaki teaches the observing portion cell 2 has a cell structure which is formed by using a spacer 11, a film which is placed between 2 glass sheets 12 and 13. The cell is provided with an inflow tube 14 of a cell culture medium and an outflow tube 15 thereof. See figure 14 and column 10, lines 45-55. The culture medium is introduced into the cell by the inflow tube 14 from a circulation pipe connected to the culture vessel, and the stage and an observing device are manipulated to observe sizes of animal cells. See column 10, lines 63-67. The culture medium containing the measured cells is returned to the culturing vessel through the outflow tube 15. See column 11, lines 5-8. There is no teaching or suggestion in Matsuzaki how to separate a cell from the other cells it is with, either physically or with markers, to be able to determine the state of the cell over time.

From all the teachings of Matsuzaki, it is clear that the culture medium with the cells is fed to an observing portion 2. There is no teaching or suggestion of a "mechanism for incubating the plurality of cells . . . in which each individual cell of the plurality of cells can be examined over time . . . ; and a mechanism for automatically tracking and identifying said individual cell over time . . . ", as found in Claim 57 and for the reasons more fully elaborated upon above in regard to Maruhashi feeding cells to an imaging device. Accordingly, Claim 57 is not anticipated by Matsuzaki and is patentable over Matsuzaki. Claims 74, 80, 86-88, 90, 93, 99, 100, 114, 115 and 118-123 are patentable over Matsuzaki for similar reasons.

The Examiner has rejected Claims 57 and 74 as being anticipated by Shuler. Applicants respectfully traverse this rejection in view of the amendments to the claims.

Referring to Shuler, there is disclosed an automated, multicompartment cell culture system. Shuler teaches an in vitro system having multicompartment cell culture system 20 having a cabinet 22. In the cabinet 22 are various cell culture chambers, where each chamber can represent a different aspect of a living being. See column 5, lines 49-53. Shuler teaches the system can be used to study the results of test chemicals that are introduced to the chambers. The test chemical is added to reservoir 58 which contains any standard tissue culture medium. Liquid is withdrawn from reservoir 50 through reservoir discharge conduit 52 by pump 54 and fed to cell culture chamber 56. Culture medium containing test chemical is withdrawn from cell culture chamber 56 through discharge conduit 58 and sensor 60 by a

pump 62. From pump 62, liquid passes through needle valve 64 and flow meters 66 before entering cell culture chamber 68. Cell culture chamber 68 can have another type of cell of the living being. Culture medium is withdrawn from cell culture chamber 68 through conduit 70 and sensor 72 by pump 74. Pump 74 returns culture medium containing test materials to cell culture chamber 56. See column 6, lines 28-35. It is clear from this, that Shuler specifically teaches to withdraw culture medium with a multitude of cells from the culture chamber 56 and feed it to sensor 60 or sensor 72 with a pump 62 and 74, respectively.

Shuler teaches that the biological and toxicological reactions/changes in cell culture chambers 56 and 68 are detected by the sensors 60 and 72, respectively, and communicate to microprocessors 98 through control lines 124a, 124b and 124c as well as input/output interface 100. A review of figure 3 clearly shows that the sensors 62 and 72 are separate and apart from the culture chambers 56 and 68, respectively. See column 7, lines 42-49. Shuler teaches that the microprocessor 98 operates the display function using continuously updated readings from sensors 60 and 72. There are no other sensors in regard to the culture chambers to monitor the cells of the culture chambers. See column 8, lines 3-8.

Shuler teaches to monitor the environment of chamber 200 through the use of ports which withdraw gas or liquid samples or introduce solid, gaseous, or liquid to this compounds or to take measurements of parameters such as pH, temperature, etc. See column 9, lines 59-64. Furthermore, the level of culture medium in container 258 is monitored using

a linear potentiometer 266 to send a signal through electrical connector 268 to the actuator for pump 234, causing that pump to increase the rate at which the medium is withdrawn from collection chamber 230. See, column 10, lines 12-19. Thus, there is no teaching or suggestion of automatically determining the state of each cell over time. Shuler teaches to facilitate withdrawal of medium having many cells from container 276, conduit 224 is provided with a dip tube 280 which will extend below the level of medium in the chamber. See column 10, lines 29-32.

Shuler teaches that once the proper temperature has been established in the culture medium, valves 64 and 80 are actuated through control lines to initiate the flow of culture medium to chambers 56 and 68, commands are sent to sensors 60 and 72 for monitoring the biological activity of the system. See column 11, lines 36-42.

In column 15, Shuler teaches all measurements are made by hand. Shuler indicates that in the third paragraph "following the 24-hour exposure period, both human lymphocytes and rat hepatoma cells were exposed to UV light source for 30 seconds. Viable cells were determined and enumerated by trepan blue dye exclusion on a hemocytometer immediately following UV exposure". The word "hemocytometer" is important because this is a manual counting system. Things are removed from the system and counted by hand. This is in no way an "automatic" bioreactor, even for counting or for manipulation based on the count. Shuler describes metabolism of toxic substances such as naphthalene.

Shuler, in every instance, teaches to feed culture medium having cells to sensors 60 and 72 which are of the flow-through type and are disposed in-line with the outflow from each compartment to thus detect, analyze and provide quantitative data regarding the test chemical effluent from each compartment. See column 13, lines 31-37. There is no teaching or suggestion of a "mechanism for incubating cells . . . in which each individual cell of the plurality of cells can be examined over time . . . ; and a mechanism for automatically tracking and identifying said individual cell over time . . .", as found in Claim 57. Since Shuler teaches to feed the culture medium with cells to the sensors from the culture chamber and not to examine each cell over time, and not automatically, Shuler does not anticipate Claim 57, and Claim 57 is patentable over Shuler. Claim 74 is patentable for similar reasons Claim 57 is patentable.

The Examiner has rejected Claims 1, 47-56, 58-64, 70, 75-79, 81, 89-92, 94-98, 103, 104, 116 and 117 as being unpatentable over Maruhashi in view of Weinreb and Shuler. Applicants respectfully traverse this rejection.

Referring to Weinreb, there is disclosed an apertured cell carrier. Weinreb teaches a perforated cell carrier 1 that includes a base 3 in which are formed apertures or holes 2. The holes are arranged in rows and columns and have a larger opening at the tops than at the bottoms. The side walls of the apertures may converge continuously or in steps towards the opening at the bottom side of the cell carrier. See column 7, lines 46-55. Weinreb teaches

the shape of the apertures 2 enables the cells to be effectively held to the carrier by applying means, such as a pressure difference between the upper and the bottom side of the carrier, or electromagnetic forces. To first separate a particular group of cells from cells of other groups, since the cells in each group are of known size or sizes, which typically differ from those in other groups, the carrier 1 is chosen to have holes of sizes so that when the matter containing the various cell groups is placed on the carrier 1, effectively most if not all of the holes are occupied by cells of the group of interest, one cell per hole. See column 7, line 63-column 8, lines 6.

Weinreb teaches to load cells into the carrier. The carrier 1 is held in place above orifice 150 and plate 152 by means of collar 154 of solution basin 156. The collar presses the carrier against the portion of plate 152 which surrounds orifice 150 and creates a seal between that portion and the carrier. This seal prevents substantial numbers of cells from passing around the edges of the carrier, rather than being captured in the apertures. Orifice 150 is connected by outflow tube 160 to pump 162. The pump serves to produce a pressure differential across carrier 1 which pulls the cells into the apertures in the carrier. A basin 156 is configured so as to allow microscope objective 158 to be brought close enough to carrier 1 so that the apertures and the carrier can be brought into focus. Solutions are provided to basin 156 by one or more inflow tubes 164 which can be connected to syringe needles 166. The inflow tubes are used to introduce various bathing and reagent solutions to basin 156. The inflow tubes are also used to wash excess cells off the top surface of carrier 1. Fluid is

removed from base in 156 by means of drain tube 170. See column 12, lines 30-64. If an electromagnetic field is desired to be used instead of a pressure difference to drive the cells into the carrier apertures and retain the cells in the apertures, the field is oriented perpendicular to the top surface of the carrier and the cells are charged accordingly. See column 15, lines 14-30. As is evident from the above description, Weinreb is interested in, and focused on separating groups of cells by filling the apertures with the desired cells of a chosen side. There is no teaching or suggestion anywhere by Weinreb of any type of a dynamically controlled environment system as found in the claimed invention. As is also evident, there is no teaching or suggestion of any automated process for determining the state of said individual cell over time, as is found in the claimed invention of applicants.

As previously explained, Maruhashi has no capability and no desire to track or capture an individual cell. The Examiner is of the position that it would be obvious to take the plate or carrier taught by Weinreb and combine it with the apparatus taught by Maruhashi. However, besides there being no teaching to combine these references, or any reason to combine these references, this position ignores the context in which the teachings of each reference is found, and that such a combination would require extensive experimentation and design and development work to even possibly approach applicants' claimed invention. For instance, nowhere is it explained how to combine a closed system with an open system or how a pressure differential or an electromagnetic field would be positioned in the apparatus taught by Maruhashi, let alone where the carrier would be placed in the apparatus of Maruhashi to

*not
specifically
taught
in prior art*

capture individual cells or even why a skilled artisan would do such a thing. Certainly Maruhashi has no desire for this, since Maruhashi is interested in tracking the shape of the cells at a given time to indicate the given state of the cells in Maruhashi. Why would Maruhashi want cells captured in the wells. There is no reason, and vice versa.

There must be some teaching or suggestion in the references themselves to combine the references, and there is none. Only the hindsight from applicants' claimed invention provides the motivation for the Examiner to combine the references, and this is contrary to patent law. The Examiner is using the applicants' claims as a road map to find each of the elements individually in different references; and having supposedly found them, the Examiner is saying the claimed invention of applicants is arrived at. However, the Examiner must take the teachings of each reference in the context in which they are found and cannot ignore this context. Weinreb is an open system while Maruhashi and Shuler are closed systems. Each of the references have their own objective, and they are incompatible with each other because of them. Weinreb is concerned with separating out a group of cells by size into individual apertures. Maruhashi is concerned with monitoring the state of a multitude of cells to note what stage they are at, and Shuler is concerned with attempting to recreate the operation of the human body by having different compartments represent different organs or portions of the body, where, like Maruhashi, Shuler is concerned with the state of a multitude of cells at a given time. In fact, this is critical for Maruhashi and Shuler because they want to know what is happening to most of the cells at a given time, and not to any one individual cell.

hindsight argument

Maruhashi wants to know when the majority of cells are of a given stage, while similarly, Shuler wants to know what a multitude of the cells are doing at a given compartment as an indication of the effects of a given material that is introduced to these cells. For this reason, no one skilled in the art would look to combine these references from the references themselves.

Just as importantly, the question arises how would one skilled in the art combine these teachings of these different references. Applicants can only guess how the closed system of Maruhashi would somehow or other be redesigned to allow the carrier of Weinreb's open system to be placed in it, and then for the pressure gradient or electromagnetic field created to cause the cells to enter the apertures. This is no small feat, since Weinreb teaches essentially only the carrier by itself in an open system and Maruhashi teaches an overall closed system that has no place for such a carrier. Moreover, Shuler teaches individual compartments in an overall closed system in which different types of cells are located in their respective compartments to simulate different aspects of a body, and again has no need, use or place for the carrier to be positioned in it. The Examiner cannot ignore this fact that there would have to be significant design, research and development to somehow or other make these systems of Maruhashi, Weinreb and Shuler compatible so the different teachings of them the Examiner is relying on can be combined.

Furthermore, all of the references are missing the important limitation of automatically tracking or determining the state of an individual cell overtime. The imaging taught by Shuler, as explained above, is performed manually, as appears to also be the case from Maruhashi and Weinreb. Weinreb refers to imaging, but it just speaks to using a microscope to look at an individual cell. Maruhashi teaches image processing, but this is to the image after it is taken and not automatically taking the image itself. There is no explanation or teaching or enablement how an automated imaging system would work. Maruhashi also does not teach or explain how an automated system would work, which yields only one conclusion, that Maruhashi and Weinreb also only teach to use manual imaging or counting since they did not explain how one would use automated imaging or counting.

Accordingly, because Maruhashi, Weinreb and Shuler are so different from each other that they cannot be combined without significant experimentation and redesign, there is no teaching or suggestion to combine them, and do not provide any type of automated tracking or determining means, Claims 1, 47-56, 58-64, 70, 75-79, 81, 89-92, 94-98, 103, 104, 116 and 117 are not obvious, and are patentable over the applied art of record.

A double patenting terminal disclaimer is enclosed to obviate the double patenting rejection.

In view of the foregoing amendments and remarks, it is respectfully requested that Claims 1, 47-64, 70, 74-81, 86-100, 103, 104 and 114-124, now in this application be allowed.

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on

Ansel Schwartz

Ansel M. Schwartz
Registration No. 30, 587

4/5/02
Date

Respectfully submitted,

JOEL S. GREENBERGER, ET AL.

By

Ansel Schwartz

Ansel M. Schwartz, Esquire

Reg. No. 30,587

One Sterling Plaza

201 N. Craig Street, Suite 304

Pittsburgh, PA 15213

(412) 621-9222

Attorney for Applicants